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Biagetti, G., Catherwood, M., Robson, N., Bertozzi, I., Cosi, E., McMullin, M. F., & Randi, M. L. (2017). HFE mutations in idiopathic erythrocytosis. *British Journal of Haematology*. <https://doi.org/10.1111/bjh.14555>

Published in:
British Journal of Haematology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
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HFE mutations in idiopathic erythrocytosis

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Introduction

Absolute erythrocytosis is characterized by persistently raised haemoglobin (Hb) and hematocrit (Ht) levels. The most studied form of absolute erythrocytosis is Polycythemia Vera (PV), a primary neoplastic disease characterized by the presence of *JAK2* mutations, risk of vascular complications and of evolution to myelofibrosis or acute leukemia. Secondary erythrocytosis is represented by rare congenital diseases (such as oxygen sensing pathway genes mutation), acquired forms or idiopathic erythrocytosis (IE). IE can be divided into two main groups. The first group consists of those with a low EPO level suggesting an unidentified molecular defect possibly involving components of the EPO signal transduction pathway. The second group is those with inappropriately normal or raised EPO level which suggests a secondary unidentified cause possibly with aberrations in the oxygen sensing pathway (McMullin, 2012). These patients are rarely observed in clinical practice, and little is known regarding their clinical characteristics, natural history and best management (Randi *et al*, 2015).

Hereditary Haemochromatosis (HH) is a disease which leads to an iron balance impairment characterized by raised serum ferritin and resulting iron overload in several organs. There are four types of HH, classified by the genetic abnormality underlying the disease. The most frequent is Type 1, associated with HFE gene mutations on chromosome 6 (Yun & Vincelette, 2015): the most common HFE mutations observed are H63D and C282Y which allele frequencies in Europe are respectively 13.6% and 3.8% (Merryweather *et al*, 1997). The HFE gene encodes for a protein involved in the regulation of hepcidin and hence iron homeostasis (Gao *et al*, 2008). Increased iron bioavailability which results from the defects of molecules involved in iron balance could lead to increased erythropoiesis and hence erythrocytosis (Camaschella, 2013), with increased levels of ferritin often observed in IE. Postulating that a possible underlying cause for erythrocytosis could be impairment in iron metabolism, we searched for the presence of HFE mutations in IE.

Patients, materials and methods

We studied 56 patients with IE: 18 were enrolled in Padua (Italy) and 38 in Belfast (UK). All the patients had normal serum EPO level and none of them carried *JAK2*V617F or *JAK2* exon 12 mutations. Congenital erythrocytosis were excluded for the absence of a familial increase of red cells and by the negative research of EPO-R, VHL, PHD2, HIF-1 α mutations. None of the patients had an overt hemochromatosis or a previous documented HFE mutation. Clinical and laboratory data of the patients are shown in Table I.

The procedures followed were in accordance with the Declaration of Helsinki and all patients gave informed written consent. We performed DNA extraction using Roche® “MagNa Pure Compact System” and the analysis of HFE genotypes using Lightcycler 480 by Roche®.

The frequency of HFE mutations among IE and the frequency in general population of UK and Italy (Merryweather *et al*, 1997) were compared using a Fisher's exact test. Means and standard deviations were compared with T test.

Results

The patients were mostly males in both the groups with a higher frequency in the Italian cohort. Patients in the UK cohort were significantly younger at diagnosis, had significantly higher haemoglobin (Hb), haematocrit (Ht), mean cell volume (MCV). Epo levels were low in both the groups, and lower in UK compared to Italian patients. Mean serum ferritin levels were higher in Italian than in UK patients with a significant difference ($p=0.02$).

Nineteen patients (33.9%) had high ferritin at diagnosis. In total, 25 patients (7 = 38.8% Italian and 18 = 47.4% from UK) were found with HFE mutations, representing 44.6% of our total IE population. Within our mutated patients, 12 (48%) had high ferritin levels. The number of patients with high ferritin levels is not different ($p=0.054$) among HFE mutated and wild type patients (Table II).

Two and 4 patients from UK were homozygous and heterozygous respectively for C282Y mutation; 1 Italian and 1 UK IE were homozygous H63D and 5 Italian and 9 UK were heterozygous H63D. In one Italian patient compound C282Y/H63D heterozygosity was found. Finally, two UK patients carried the S65C mutation (1 heterozygous and 1 compound S65C/C282Y).

Discussion

Still now a number of patients with high haemoglobin levels are considered affected by "idiopathic erythrocytosis", which means that the etiology of their condition is not understood. Even if it has been evaluated that about 70% of all erythrocytosis are IE (Bento *et al*, 2015), this condition is poorly studied and no proper diagnosis can be made.

In the past, a correlation among erythrocytosis and HH has been suspected (Raphael *et al*, 1979). HH patients do not always have increased ferritin levels and the presence of high ferritin is not always related to the different HFE genotypes (Olynyk *et al*, 1999), even if C282Y mutation seems to display more often an important iron overload (Burke *et al*, 2000).

Our data show that a relevant number of patients with IE carry HFE mutations in two distinct European cohorts. This observation is limited by the small number of patients, however, comparing the incidence of HFE mutations in IE and its frequency in general population (Merryweather *et al*, 1997), we found that IE patients carry H63D or C282Y more often ($p=0.048$). This represents a novel finding not previously reported.

High ferritin levels were observed in half of our mutated and in one third of wild-type patients. The difference was not significant even if there is a trend towards a higher frequency of high ferritin in mutated patients. Probably, a statistical difference could become evident exploring a higher number of patients.

Unfortunately, transferrin saturation, that is now considered an important criterion for hemochromatosis diagnosis (Bacon *et al*, 2011), was not collected in most patients at the time of diagnosis together with ferritin values. Many of the patients included in this study have undergone

phlebotomies (about 60% of the patients) for the treatment of erythrocytosis, and for this reason we considered to be useless a later transferrin saturation evaluation.

Not surprisingly, HFE mutations were more frequent in UK cohort, and there is a particular frequency of C282Y mutation: this is compatible with the finding of the founder effect in Celtic population (Lucotte & Mercier, 2000). Interestingly, we encountered in one Italian patient the presence of a C282Y mutated allele (compound C282Y/H63D heterozygosity). This genotype is a rare finding in southern-Europe (Merryweather *et al*, 1997; Cassanelli *et al*, 2001). In 2 patients, both from UK, we found a S65C allele: in one case it was compound with C282Y and in one case it was heterozygous. The allele frequency of S65C is rare and in white population is estimated to be 1.6% (Beutler *et al*, 2000).

The mechanism of IE in patients with HFE mutations has to be elucidated. While an iron overload can be claimed in patients with raised ferritin levels, the relationship between IE and HFE when ferritin is within normal levels is not yet understood. HFE mutated protein limits hepcidin expression and increases transferrin-bound iron uptake by erythroid cells.

It has been demonstrated that HFE C282Y homozygotes display increased plasma iron turnover and increased erythropoiesis suggesting that HFE mutations alter the supply of iron to the erythroid tissues (Feeney *et al*, 2005). In this work, we searched for a possible new cause for erythrocytosis, and our findings suggest a possible relation between the presence of HFE mutations and erythrocytosis, although hepcidin, ferroportin, erythroferrone and other molecules involved in iron regulation have to be evaluated in large cohort of IE patients to clarify the physiological relation between HH and erythrocytosis.

Table I: Main clinical and laboratory data of patients with erythrocytosis.

	ITALIAN COHORT	UK COHORT	TOTAL
N°	18	38	56
Males (%)	17 (94.4)	27 (71.0)	44 (78.6)
Mean Age at diagnosis \pm SD (years)	54 \pm 12	44 \pm 14	47 \pm 14
Mean Hb at diagnosis \pm SD (g/dL)	17.8 \pm 1.0	19.6 \pm 1.9	19.0 \pm 1.8
Mean Ht at diagnosis \pm SD (%)	52 \pm 3	57 \pm 7	55 \pm 6
Mean WBC at diagnosis \pm SD	8.0 \pm 2.5	8.3 \pm 2.2	8.1 \pm 2.3
Mean MCV at diagnosis \pm SD ($\times 10^9$ /l)	88 \pm 5	96 \pm 7	94 \pm 7
Mean Plts at diagnosis \pm SD ($\times 10^9$ /l)	228 \pm 53	220 \pm 71	223 \pm 65
Mean EPO at diagnosis \pm SD (UI)	10.0 \pm 5.7	5.9 \pm 2.6	7.0 \pm 4.1
Mean Ferritin at diagnosis \pm SD (ng/ml)	330.5 \pm 239.2	162.9 \pm 146.1	227.7 \pm 202.5

Table II: Different HFE genotypes and their distribution among patients with high or normal ferritin levels

	High ferritin	Normal ferritin
N° of patients	19	37
H63D/H63D (%)	1 (5.3)	1 (2.7)
H63D/wt (%)	8 (42.1)	6 (16.2)
C282Y/C282Y (%)	1 (5.3)	1 (2.7)
C282Y/wt	1 (5.3)	3 (8.1)
H63D/C282Y	1 (5.3)	0
C282Y/S65C	0	1 (2.7)
S65C/wt	0	1 (2.7)
Wt/wt	7 (36.8)	24 (64.9)

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Authorship

GB Analysed the data, wrote the paper and performed the molecular analysis. **MC** and **NR** performed the molecular analysis. **IB** contributed clinical data, followed the patients and carried out the statistical analysis. **EC** contributed clinical data and followed the patients. **MFM** and **MLR** designed the study and critically reviewed the manuscript. All Authors have read and approved the final version.

Disclosure

The authors have no conflicts of interest to disclosure.